

Clean Version of Amended and New Claims contained  
in Applicant's AMENDMENT AND REPLY TO FEBRUARY 14, 2002  
OFFICE ACTION as required by 37 CFR 1.121 (c)(1)(i)

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1. A method of extracting lipid associated sialoprotein from the following body fluids, cerebrospinal fluid, urine, saliva and sputum and determining the amount of lipid associated sialoprotein in a sample of such fluid which comprises the following steps:

a) adding to the sample a mixture of a chlorinated lower alkyl hydrocarbon and a lower alkyl alcohol;

b) mixing the resulting admixture for a suitable period of time to dissolve lipid-bound sialic acid in the sample in the chlorinated hydrocarbon/alcohol mixture;

A c) centrifuging the mixture at high speed to form a substantially clear upper phase;

d) separately recovering from the clear upper phase so formed a predetermined volume of the upper phase;

e) adding to the predetermined volume of the upper phase an amount of a mixture of an aqueous protein-precipitating agent without any adsorbing material, the amount of mixture being effective to cause precipitation of the lipid associated sialoprotein;

f) vortexing the resulting admixture;

g) centrifuging and recovering the resulting precipitate;

h) washing the precipitate in a saline solution;  
i) centrifuging the resulting mixture;  
j) dissolving the precipitate in water;  
k) adding to the solution a hydrolysis agent;  
l) heating the resulting admixture;  
m) determining the amount of lipid associated sialoprotein present in the solution and thereby the amount present in the fluid sample. (AMENDED).

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6. A method according to claim 1, wherein in step (c) the mixture is centrifuged at about 6000 rpm for at least 5 minutes. (AMENDED).

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17. A method of diagnosing cancer in a human subject which comprises determining the amount of lipid associated sialoprotein in a sample of the subject's cerebrospinal fluid, saliva or sputum according to the method of claim 1 and comparing the amount so determined with amounts previously obtained for subjects known to have cancer by use of the method of claim 1. (AMENDED).

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18. A method of diagnosing cancer in a human subject which comprises determining at regular time intervals the amount of lipid associated sialoprotein in a sample of the subject's cerebrospinal

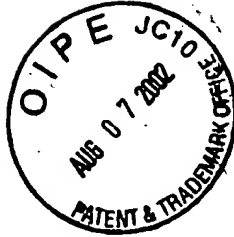
fluid, saliva or sputum sample according to the method of claim 1 and comparing the amounts so determined with amounts previously obtained for the subject by use of the method of claim 1. (AMENDED).

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19. A method of diagnosing cancer in a human subject by applying the method of claim 17 to a sample of the subject's peritoneal fluid, pleural fluid or bronchial washings. (NEW).

A<sup>4</sup> 20. A method of diagnosing cancer in a human subject by applying the method of claim 18 to a sample of the subject's peritoneal fluid, pleural fluid or bronchial washings. (NEW).

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I hereby certify that the foregoing **RESPONSE TO JULY 19, 2002 OFFICE COMMUNICATION** with respect to the application described below together with the required fee are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service on **August 7, 2002** and is addressed to the U.S. Department of Commerce, Patent and Trademark Office, Organization - TC 1600, Bldg./Room CM1, Washington, DC 20231.

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**Title:** **METHOD FOR DETERMINING LIPID ASSOCIATED SIALOPROTEIN IN BODY FLUIDS**

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**Attorney Docket No.:** **NK-3**

**Application No.:** **09/802,457**

**Art Unit** **1642**